Relative Adjuvant Efficacy of $Al(OH)_3$ and Saponin Is Related to the Immunogenicity of the Antigen

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$Al(OH)_3$ is the most widely used adjuvant in human and veterinary vaccines [1]. Saponin, a mixture of triterpene glycosides extracted from the bark of the Quillaia tree [2], is included in foot-and-mouth disease vaccines [2] and is also very effective in experimental vaccines against protozoal parasites [3]. The two adjuvants differ markedly in their relative efficacy depending on the antigen. The humoral response to bovine serum albumin (BSA) is better potentiated by $Al(OH)_3$ than saponin, whereas the opposite is the case for sheep red blood cells (SRBC) [4].

This differential adjuvant activity may be an expression of underlying differences in the mechanism of action of the adjuvants. It could depend on the intrinsic immunogenicity of the antigen, SRBC being potent immunogens, whereas BSA is readily tolerogenic [5]. This communication presents the results of experiments designed to provide further evidence for this hypothesis, using as antigens BSA and keyhole limpet haemocyanin (KLH), and DNP conjugates of BSA, fowl gamma globulin (FGG) or KLH. BSA, FGG and KLH represent an ascending series of antigenic strength in terms of their ability to elicit T-cell mediated help [6].

The potentiation of the primary humoral response to BSA or KLH by $Al(OH)_3$, saponin, or a mixture of the two adjuvants was compared (table I). The effect of combining the adjuvants was tested in order to find out if BSA would become susceptible to the adjuvant action of saponin if it were insolubilized by adsorption onto $Al(OH)_3$. Saponin does not interfere with the uptake of ^{125}I-BSA by $Al(OH)_3$ (data not shown). The selective efficacy of $Al(OH)_3$ for BSA was confirmed, but its adjuvant effect was inhibited rather than enhanced by the addition of saponin. In contrast, the response to KLH was better potentiated by saponin than $Al(OH)_3$ and the two adjuvants interacted positively rather than negatively.

Table I. $Al(OH)_3$ and saponin as adjuvants for KLH and BSA

Immunization	Day 28 Ab response	
	anti-KLH (RIA endpoint)	anti-BSA (log_{10} ABC, mean ± SD, n = 5)
Antigen	10	−0.53 ± 0.54
Antigen + $Al(OH)_3$	80	1.33 ± 0.31
Antigen + Saponin	640	−0.35 ± 0.43
Antigen + $Al(OH)_3$ + Saponin	1,280	0.14 ± 0.44

Mice were immunized s.c. with 2.5 μg KLH (Pacific Biomarine Labs.) or BSA (Armour) alone or plus 100 μg (of Al) $Al(OH)_3$ (alhydrogel, Superfos) or 50 μg saponin (Food Industries Ltd.). The responses was measured by a solid phase radioimmunoassay [8] for KLH and the Farr test [9] for BSA.

The comparison of $Al(OH)_3$ and saponin was extended to the humoral response to DNP coupled to BSA, FGG or KLH. The relative efficacy of the adjuvants depended on the carrier, $Al(OH)_3$ being superior to saponin with DNP-BSA and DNP-FGG, and the opposite with DNP-KLH (table II). The effect was not correlated with the concentration of antigen or the titre of anti-DNP antibody. Thus, the response to DNP-KLH remained selectively susceptible to saponin even when the dose of DNP-KLH was reduced to 0.1 μg, at which level the anti-DNP response (plus saponin) was very close to that elicited by 1 μg of DNP-BSA or DNP-FGG plus $Al(OH)_3$, and considerably less than that with 10 μg of the latter antigens.

These results strengthen the evidence in favour of the hypothesis that the selective adjuvant efficacy of $Al(OH)_3$ and saponin depends on the immunogenici-

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e II. Relative efficacy of $Al(OH)_3$ and sa[redacted]in as adju- DNP-BSA, DNP-FGG and DNP-KLH

	Dose μg	Day 28 antibody response (RIA endpoint)		
		no adjuvant	$Al(OH)_3$	Saponin
SA	1	<10	320	<10
	10	10	<5,120	1,280
GG	1	<10	640	<10
	10	10	<2,560	80
LH	0.1	>10	10	640
	1	>10	40	2,560
	10	160	2,560	20,240

P-BSA (11 DNP/mol), DNP-FGG (12 DNP/mol) or DNP- 1 DNP/10^6 daltons) were injected s.c. alone or plus 100 μg $Al(OH)_3$, or 50 μg saponin. The response was measured by iase radioimmunoassay [8], using DNP-BSA plates for the NP-FGG and DNP-KLH sera, and DNP-KLH plates for -DNP-KLH sera.

he antigen. In themselves, they do not complete- lude a role for the molecular weight (MW) of the :n, since KLH (MW > 10^6) is larger than BSA 50,000) or FGG (MW 160,000). However, re-)bservations using human growth hormone as :n enable this factor to be discounted; $Al(OH)_3$ ot saponin potentiated the humoral response in esponder CBA mice, whereas saponin is the bet- ljuvant in high responder Balb/c mice (*Bomford* , unpubl.). The physical nature of the antigen l still be important for the adjuvant action of nin for SRBC and other cellular antigens which ain membrane cholesterol, to which saponin can forming a highly immunogenic complex [7]. ıe recognition that differences in relative adju- activity are related to antigenic strength offers a basis for the investigation of the mechanism of vant action, and is also important in the selection ljuvants for experimental immunization or vacci-)n.

References

1 Aprile, M.A.; Wardlaw, A.C.: Aluminium compounds as adjuvants for vaccines and toxoids in man: a revi w. Can. J. publ. Hlth *57:* 343–360 (1966).

2 Dalsgaard, K.: A study of the isolation and charact rization of the saponin Quil A. Evaluation of its adjuvant activity, with a special reference to the application in the vaccination f cattle against foot-and-mouth disease. Act. vet. scand., suppl. 69, pp. 1–40 (1978).

3 McCohn, A.A.; Bomford, R.; Dalton, L.: A comparison of saponin with other adjuvants for the potentiation of protective immunity by a killed plasmodium yoelii vaccine in the mouse. Parasite Immun. *4:* 337–347 (1982).

4 Bomford, R.: The comparative selectivity of adjuvants for humoral and cell-mediated immunity. I. Effect on the antibody response to bovine serum albumin and sheep red blood cells of Freund's incomplete and complete adjuvants, alhydrogel, *Corynebacterium parvum*, *Bordetella pertussis*, muramyl dipeptide and saponin. Clin. exp. Immunol. *39:* 426–434 (1980).

5 Mitchison, N.A.: Induction of immunological paralysis in two zones of dosage. Proc. R. Soc. gen. Microbiol. *161:* 275–292 (1964).

6 Mitchison, N.A.: The carrier effect in the secondary response to hapten-protein conjugates. I. Measurement of the effect with transferred cells and objections to the local environment hypothesis. Eur. J. Immunol. *1:* 10–17 (1971).

7 Bomford, R.: Studies on the cellular site of action of the adjuvant activity of saponin for sheep erythrocytes. Int. Archs Allergy appl. Immun. *67:* 127–131 (1982).

8 Scott, M.T.; Moyes, L.; Wood, J.N.: Lack of identity between the 90K protective glycoprotein of Trypanosoma cruzi and hybridoma (CE5)-defined *T. cruzi* antigen which crossreacts with mammalian neurones. Trans. R. Soc. trop. Med. Hyg. *76:* 698–700 (1982).

9 Bell, E.B.; Shand, F.L.: Cellular events in protein tolerant inbred rate: the fate of thoracic duct lymphocytes and memory cells during tolerance induction to human serum albumin. Eur. J. Immunol. *3:* 259–267 (1973).

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